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NOTES ON REGENERATION.

T. H. MORGAN.

During the past summer I made at Woods Holl a number of observations and experiments on the regeneration of several animal forms. The results are here brought together, although they have little more in common than that they all deal with problems of regeneration.

The Limitation of the Regenerative Power of Dendrocœlum lacteum.

The fresh-water planarians show such remarkable powers of regeneration that it is surprising to find in one of them, *Dendrocœlum lacteum*, that this power is much reduced. The question at once arises whether we can discover anything peculiar in the relation of this planarian to its surroundings, or in its internal structure that will give a clue to its exceptional behavior.

There is nothing in its habitat to suggest that it has lost, or has never acquired to the same degree, the power of regeneration possessed by other planarians. In the pond at Falmouth where I collected this species there were also present, sticking to the under surfaces of the same stones, both *Planaria maculata* and *Phagocata gracilis*. If *Dendrocœlum* is not as subject to injury as are the other two species, and if, therefore, it does not need the same regenerative power, it is remarkable that *Dendrocœlum* should be so uncommon in comparison with the other two forms. If it is subject to greater injury, then it has not acquired the power to meet the situation as have the other species. Considerations of this kind do not have, I believe, any real bearing on the question of whether an organism has or has not acquired the power to regenerate, although some biologists lay great stress on this sort of speculation. The limitations in the power of regeneration of *Dendrocœlum* are peculiar. Lillie found that when only

the anterior end of the worm is cut off a new anterior end is regenerated. This power to produce a new head was found to extend back to about one-third of the length of the worm, *i. e.*, to a region just in front of the pharynx. Behind this level the posterior piece fails to regenerate a head at its anterior end.

On the other hand, the anterior pieces regenerate a new posterior end from any level, with the possible exception of the immediate region of the head itself; but the latter point has not yet been sufficiently examined in this species. It appears a remarkable fact that this planarian should have such extensive powers of regenerating posteriorly, and such limited powers of regenerating anteriorly, especially since, as far as we know, the same cells produce either a head or a tail according to which end is exposed; but this has not been definitely determined, and would be almost impossible to determine with absolute certainty. Eugen Schultz has also studied the regeneration of *Dendrocœlum lacteum* of Europe[1] and finds that posterior pieces do sometimes regenerate a head, although the regeneration is very slow, and it may appear that Lillie did not keep his pieces a sufficiently long time for the regeneration to take place. He states, in fact, that most of the posterior pieces died after five or six days. Schultz believes that these posterior pieces have potentially the power to regenerate, but that sometimes the piece closes in such a way that the formation of new tissue is prevented, as I have found to occur occasionally in *Bipalium*. Lillie, on the other hand, tries to account for the lack of power of posterior pieces to form a head by means of the following hypothesis. He suggests that the regeneration from the posterior cut surface at all levels is due, in some unexplained way, to the presence "of the brain and anterior part of the nervous system in the anterior piece." Conversely the absence of these structures in posterior pieces is supposed to account for the lack of regeneration from the anterior cut surface. A simple experiment would have shown the untenability of this point of view. If the head end is cut off just in front of the pharynx so that the brain and the anterior part of the nervous system are removed, and then the tail end of the middle

[1] It has been assumed that the European *Dendrocœlum lacteum* and the American form or forms are identical, but I think this question will bear further examination.

piece is also removed, it will be found that the middle piece without regenerating a new head will still regenerate a new tail. This shows conclusively that Lillie's supposition in regard to posterior regeneration is erroneous. The remainder of his argument, which rests on this assumption, also falls, I believe, in the light of this fact.

The great mortality that Lillie observed in the posterior pieces is due largely, at least in my experiments in which the same thing was observed, to the temperature being too high, or possibly to exposure to light. If the pieces are kept cooler (by surrounding the dishes by the cool, running salt water of the laboratory) the mortality is much reduced, and instead of dying after six days, as in Lillie's experiment, I have kept short posterior pieces for several weeks. It is only by keeping such pieces for a long time that one can fairly test their powers of regeneration.

Schultz states that he cut *Dendrocœlum* in two either between the pharynx and the reproductive region or else in front of the pharynx. In the former case he found that the posterior pieces regenerated an anterior end very slowly, and he found it more profitable in studying the regeneration of the head to use those posterior pieces that had been cut off in front of the pharynx. He found that the regeneration of the anterior end often failed to take place, and he attributes this to fusion of the sides of the cut surfaces, as I had found to occur not infrequently in *Bipalium*. Whether this is the whole of the question remains to be seen. In a marine polyclad, *Leptoplana*, Schultz found that posterior pieces, no matter at what level they have been removed, fail to regenerate an anterior end, even when only a small piece of the head is cut off. Yet regeneration from a posterior cut surface takes place at all levels. Schultz attributes the lack of regeneration at the anterior end either to the closing over of the "growing point" by the coming together of the old tissue from the sides, or to the muscles from the sides uniting and thus preventing further growth. Both factors he thinks may enter into the result. This point could be tested, I think, by making the cuts so that there is left a pointed anterior end, when regeneration should occur, if Schultz's view is correct. From an experiment of this sort that I have carried out on *Dendrocœlum* I think it

probable that in *Leptoplana* also no better regeneration would occur, even at a pointed end,[1] and if this proves to be the case Schultz's explanation is insufficient.[2]

In my experiments I first examined whether the form of the cut surface at the anterior end had anything to do with the lack of regeneration, for it was possible here, as in the case of *Bipalium*, that the cross-cut surface closed in such a way that subsequent regeneration was prevented. By changing the form of the cut surface this difficulty should be eliminated. Posterior pieces were cut off through the region of the pharynx and also behind the pharynx. The anterior ends of some of these pieces were very oblique; others were pointed in the middle, *i. e.*, they were cut off by two oblique cuts meeting in the middle line. In the latter case especially it is impossible that the muscles from the sides could close the anterior cut surface.[3] These pieces were kept alive for two or three weeks, and although it could be seen that there was a little new tissue at the anterior cut surface, yet no further regeneration occurred after the first ten days or thereabouts, and there is no indication that regeneration would have gone any further if the pieces had been kept alive for a greater length of time.

Sections of these pieces were made. The results will be given below.

In two other series each worm was cut into three pieces. The head pieces extended to the middle of the region in front of the pharynx. These pieces should be capable of regenerating at the

[1] Loeb says that *Thysanozoon* regenerates a new head, but he did not determine whether a new brain is formed. Monti also obtained regeneration in this form *and also in Leptoplana*, except when cut far posteriorly. Lang also records regeneration in marine polyclads.

[2] Schultz states in the opening of his paper that I carried out my experiments without making sections of the planarians, and he intimates that had I done so I would not have reached certain conclusions in regard to the growth of the new part. How Schultz obtained this information it would be interesting to know. Probably he based his generalization on the absence in my earlier papers of reference to histological details with which I was not then especially concerned. As a matter of fact I had made and studied many sections. My students also were at work on the minute anatomy, and one of them published a complete account of the histological changes taking place during regeneration before Schultz's paper appeared.

[3] Whether union of the dorsal and ventral muscles might close these pieces I have not here considered.

posterior end. The middle pieces included the next portion of the worm, and extended to the region of the reproductive pore. These pieces should be capable of regenerating a head at their anterior ends and a tail at the posterior ends. The third pieces were the tail pieces and included the rest of the worm. These pieces should be incapable of regenerating a head at the anterior end. The pieces were preserved at intervals of 1, 2, 3, 4, 5, 6 days, killed, embedded, stained and examined with immersion lenses.

A study of the sections shows that the changes taking place at the anterior end of the tail-pieces appear to be similar in all respects to those that occur at anterior or posterior surfaces at which regeneration of the missing part takes place. There is nothing in the sections to show why the regeneration should continue in the one case and not in the other, and it is difficult to believe from the evidence of the sections that anterior regeneration from the tail-pieces would not in time be accomplished, yet after three weeks there was no sign of further regeneration and I am forced to conclude with Lillie that in the form of *Dendrocœlum* found at Falmouth regeneration does not, ordinarily at least, occur behind the level of the pharynx. Sections through tail-pieces, cut off behind the pharynx and kept for nearly three weeks, show that the formation of new tissue has not gone much beyond that of the first six days, and that a new head has not been produced. Sections of the oblique, and of the pointed tail-pieces give exactly the same results.

Several writers seem inclined to account for the lack of regeneration in certain planarians, and especially from the posterior region of the body, as due to the absence or small size of the nerve cords in these regions. With this view I do not agree. Lillie has used *Dendrocœlum* as a case in point. Sections of this worm show, however, that the cords in the more posterior regions are as well developed, judging from their size, as they are in *Planaria maculata*.

Regeneration in Pycnogonids.

In 1895 Loeb published some observations that he had made on the regeneration of one of the Pycnogonida, *Phoxichilidium*

maxillare. He cut the animal in two between the second and third pairs of legs, and found in two cases that after a time a new part suddenly appeared, presumably after a moult. This new part that regenerated at the posterior end of the anterior piece Loeb speaks of as a body, and points out that this is the first case observed in the arthropods in which new body segments have been seen to regenerate. I have repeated this experiment during two summers, for it did not appear to me beyond dispute that the new part that had been observed was necessarily a body, since no satisfactory evidence that it was such is furnished by Loeb's paper. Although sections of the new part were, apparently, made, no posterior opening of the digestive tract was found, no ganglia are described as being present in the new body, nor do new legs appear to have been present at the sides as we should expect if this new part were really a body.

My first experiments were made in 1901 and, although a number of pycnogonids were kept for two months or longer, none of them regenerated at the posterior end. Since I had used large individuals it seemed not improbable that the lack of regeneration might have been connected with the maturity of the individuals. During the past summer I have repeated the experiment on a large scale, both with large and with small individuals; but although many of the pieces were kept for nearly two months no regeneration took place, with the possible exception of two instances that will be described.

In a number of cases the individuals were cut in two between the third and fourth pairs of legs, *i. e.*, nearer the posterior end than Loeb had cut them, for, from analogy with other cases, it seemed more probable that if the body could regenerate at all it would be more likely to do so the nearer the cut was made to the posterior end. Other individuals were cut in two between the second and third pairs of legs. In only one case did regeneration appear to take place, as shown in Fig. 1. Here the bases of the fourth pairs of legs bulge out as though they had been formed anew, and it seems possible that the rudimentary abdomen is also new, although it is also possible that a part at least of this structure had been left unintentionally when the cut was made. Sections show that the digestive tract opens at the

end of the abdomen. There is no trace of further regeneration within the stumps of the legs. At most, the bases of the legs and the abdomen, or part of the latter, have regenerated.

FIG. 1.

The second case is shown in Fig. 2, in which there is only a bulging out of the end of the body. The cut had been made in this case between the second and third pairs of legs. Sections of this individual do not show any indications of the development

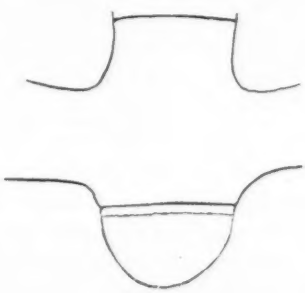


FIG. 2.

of legs or of a rudimentary abdomen in the new tissue of the bulging portion, and there is nothing to indicate that the development would ever have gone any further. The digestive tract ends blindly and is not connected with the ectoderm.

In looking over a large number of individuals I found a few cases in which a leg on one side was much smaller than its

opposite, and from this it seems probable that the original leg had been lost at the breaking joint at the base, and a new one had begun to regenerate. Moreover, I found one case in which the new leg was clearly a new structure, Fig. 3. The different segments had not yet been formed in their adult proportions and

FIG. 3.

the leg could not have been functional as yet. There is some resemblance between this leg and the newly regenerated part from the posterior end of the body that Loeb saw and figured. In fact this idea seems to have suggested itself to Loeb for he writes: "Das Vorhandensein eines ueberzähligen Segmentes könnte vermuthen lassen, dass das neugebildete Stück vielleicht im Laufe der Zeit sich zu einer Extremität entwickelt haben würde, dass es sich also um die Bildung eines Beines an Stelle des abgeschnittenen Rumpfstückes gehandelt habe, ein Fall, den ich als Heteromorphose bezeichnete. Allein Hoek führt an, dass bei Ammotheen das Abdomen nicht selten Spuren einer Segmentation zeigt." Thus in order to explain away the presence of too many segments in the new part Loeb has recourse to a condition found in another species — a mode of explanation that will scarcely recommend itself.

A somewhat fuller analysis of these two cases of Loeb's may not be unprofitable. If the new part is really a body, *i. e.*, thorax and abdomen, we should expect to find the digestive tract opening at the posterior end, but this does not appear to have been the case, for, Loeb says: "Der Darm setzte sich in den vorderen Theil des regenerirten Stückes fort. Im Uebrigen aber waren die Gewebe wenig differenzirt." It is to be remembered that the digestive tract also continues out into the legs in the pycnogonids as a blind sac. In the second place, while the three segments of his first example might be interpreted as representing the two remaining thoracic segments, and the rudimentary abdomen, yet in the other case five or six segments appear in the new part. It is this that led Loeb to suggest that the new part

might represent a leg; but he withdraws this interpretation at once as seen above. There is certainly no striking resemblance between the new part figured by Loeb and the abdomen of *Ammothea*. Finally, if the new part is a new thorax where are the legs?

In the light of these considerations we must wait until some one, favorably situated, has an opportunity to work over the subject with ample materials. Meanwhile it seems to me that so far as the evidence goes it is rather in favor of the view that the regeneration described by Loeb is a new leg and not a part that replaces the lost segments of the thorax and abdomen.

The Lack of Regeneration of the Pigment Spot in the Fin of Fundulus.

If a gold fish having a black band at the end of its tail be selected, and the end of the tail be cut off proximal to the band, a new band like the one removed reappears in the regenerated tail. The presence of black pigment at the cut surface from which the new part regenerates is clearly not necessary for the development of pigment in the new part. This result is all the

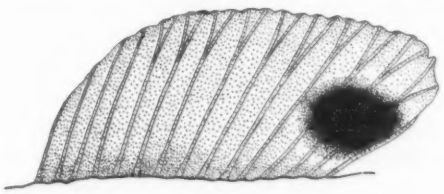


Fig. 4.

more curious since the occurrence of the pigment band is only an individual peculiarity. It seemed desirable to try the same experiment in a species in which a characteristic spot or a ring was present. The dorsal fin of the male of *Fundulus majalis* has a black spot in its posterior part, Fig. 4. The spot is not present in the female, and it appears, therefore, that this color marking belongs to the category of secondary sexual characters.

The posterior part of the fin was cut off by an oblique cut; the part removed containing all of the black spot. The lost part was slowly replaced, and in the course of two months the fin was completed, but the pigment spot did not come back, and there

was no evidence that it would have done so if the fish had been kept longer. Since the operation had been carried out during the height of the breeding season, it seemed possible that the spot might normally fade out later, but other fish, examined in September, showed the spot still present.

The results on *Fundulus* appear to be different from those on the gold fish, and it is not apparent why this difference should exist. The result does not seem to be connected in any way with the fact that the spot in *Fundulus* is a secondary sexual organ. The most plausible explanation that suggests itself is that in the tail of a gold fish that has a black tip there are cells throughout the tail that can develop pigment should they get into the terminal portions of the tail, while no such cells are present in *Fundulus*, or if present they fail to produce pigment in the new part. It may be that in *Fundulus* all the cells capable of producing pigment have been already carried into the pigment-spot itself, and hence when this spot is removed no cells capable of developing this pigment are present in the remaining part. Further work will be necessary to determine whether these suggestions have any value.

The Method of Closure of the Cut Ends of Tubularia.

The peculiar method of closure of the cut ends of *Tubularia* has attracted attention since it appears to be different from the closing observed in other forms. I have already discussed at some length this process[2] and shall not repeat here what has been already said, but since I have observed during the past summer certain processes that seem to throw some light on this question I shall briefly refer to them in this connection. Stevens has figured the closed end of a piece that had been cut through the hydranth-forming region at the time when the primoidium of the new hydranth had just been laid down, and when the red pigment lines, that indicate the appearance of the new hydranth, were present. Over the closed end the red lines radiate to the center of the bounding membrane. It seemed to me that a further examination of pieces that closed in this way might throw some light on the process in general. Pieces were cut off and

[1] Morgan, *Roux's Archiv*, XIV., 1902.
[2] "Regeneration," 1901, p. 69.

kept about twenty-four hours when the primoidia of the new tentacles had begun to appear. At this time I cut the ends squarely off, the plane of section lying across the middle of the new proximal tentacles. To my surprise the cut ends now closed in a very different way from that of ordinary cross-cut pieces. The whole wall contracted from the perisarc and the cut edges were brought together almost at once, and subsequently fused, often showing the radiating lines described by Stevens over the new end. It was perfectly clear that the result was due to a contraction of the cœnosarc, and the difference between this process and that shown by ordinary pieces appears to be due entirely to the fact that at the time when the tentacle primoidia are laid down, the cœnosarc has become free from the outer wall, or perisarc.

From this result it seems to me to follow with great probability that in ordinary pieces the closure of a cut end is also due to a process of contraction of the cœnosarc, but ordinarily the wall of the cœnosarc is so closely stuck to the inner surface of the perisare that it is not free to pull away as a whole, and there is a consequent drag that holds back the contracting wall, and a consequent modification of the method of closure of the opening. This conclusion also fits in well with some facts observed at the time of closure of the pieces. Certain of the cells that appear to be more closely stuck to the wall are often left behind, or are retarded in their progress towards the center of the newly forming membrane. Thus the peculiar method of closure of *Tubularia* finds its explanation in the unusually close connection between the perisarc and cœnosarc. I have tried to show elsewhere[1] that this same connection may also be responsible for the characteristic "incomplete structures" of *Tubularia*, whose chief peculiarity is that their organs are full sized so far as they are formed.

Transpositional or Compensatory Regeneration of the Large Chelæ in some Crustacea.

Przibram[2] discovered in 1901 in the decapod *Alpheus* that it is possible to cause the small claw (chela) of one side to become

[1] "Some Factors in the Regeneration of Tubularia," *Roux's Archiv*, XIV., 1903.
[2] *Roux's Archiv*, XI., 1901.

the large claw by the simple operation of removing the large claw of the other side. At the next moult the small claw becomes the big one, and the newly regenerated claw becomes the small one. Zeleny[1] found in 1902 that a similar throwing over of the large operculum of the annelid, *Hydroides*, can be brought about by the same sort of operation. Wilson[2] in 1903 made some important additions to Przibram's work, using an American species of *Alpheus*. He suggested that the small claw is merely an arrested stage of development of the big claw, and that when the big claw is removed the check is at the same time taken away that holds back the development of the small claw. At the next moult the small claw becomes the large one, and the new claw the small one.

As yet no one has detected the nature of the correlation that causes the transposition, and this must obviously be the next step in advance. Wilson has suggested that the throwing over is connected with the nervous system, but the experiments on which he bases this suggestion appear to me to be capable also of another interpretation.

During the past summer I undertook some experiments which I hoped would give results bearing on this question, but the outcome has been almost entirely negative. Nevertheless, I shall venture to describe these experiments briefly, because if carried out on more suitable forms they will very probably throw some light on this exceedingly important subject.

Several years ago I found that by cutting the nerve of the leg of the hermit-crab, proximal to the breaking joint, the leg can then be cut off at any level beyond the breaking joint without the remaining part being thrown off at the base. By removing portions of the large leg at different levels, after first cutting the nerve at the base, I hoped to be able to discover whether the amount removed had any effect on the transposition of the large claw to the other side. It was also possible that the simple cutting of the nerve might have some effect, as Wilson's experiment seems to show. The result might also, as Wilson appears to believe, depend in part upon the degree to which the new nerve

[1] *Roux's Archiv*, XIII., 1902.
[2] BIOLOGICAL BULLETIN, IV., 1903.

regenerated before the next moult. In practice, however, it is not possible to cut the nerve without cutting also the blood-vessels, and the injury to the latter may be as important as, or even more so, than that to the nerve.

The experiments were carried out with the hermit-crab and with the fiddler-crab, but were unsuccessful in both cases for different reasons. First, the transposition does not occur under any circumstances in the hermit-crab, as this and other experiments showed; and second, in the fiddler-crabs the muscles, etc., beyond the breaking joint degenerate after the operation. This caused the death of most of the crabs, and those that remained alive had only the outer shell of the leg beyond the breaking joint, and even this fell off in several cases. Since, however, the operation can be carried out in the hermit-crab without the outer part of the leg degenerating, it may be possible, in other forms that have the power of transpositional regeneration (in *Alpheus*, for example), to carry out this experiment successfully.

In both the hermit- and the fiddler-crab I also tried the effect of removing three of the walking legs on the same side of the body as the big claw, leaving the big claw uninjured, in order to see if the absence of the other legs might possibly affect the transposition. This did not succeed, because in the hermit-crabs, as I have said, the big claw does not throw over, and in the fiddlers the experiment had to be brought to an end before any of the crabs had moulted.

All of the individuals of the hermit-crab that I have examined were right-handed, and the shells in which they live have also right-handed spirals. It has been suggested to me that this is an adaptation, in so far that the right-handed hermit crab is placed to better advantage in a right-handed shell. Consequently, if this were true (and I am by no means certain that it is so), it would be disadvantageous for the hermit-crab to have the power of transposition after the loss of the big claw, and in consequence this power has not been acquired, or else, if it existed in the ancestors of the hermit-crabs, it has been lost. That there is really no basis for an argument of this kind is shown by the state of affairs in other decapods; in the lobster, for example. In the American lobster I have seen several cases in which the

big claw had been lost and a new one of the same kind was regenerating on the same side. Przibram has also described cases of this sort. This result is all the more interesting since in the lobster the big claw is present in some individuals on one side, and in other individuals on the other. It cannot be claimed in the lobster that one kind of claw represents an undeveloped stage of the other. In the regeneration of the claws, as especially well seen in the lobster, the particular type of claw is present, although not always fully developed, at an early stage, as Przibram has described, and as I have also found. No doubt the advocates of the view that all beneficial processes have been acquired because of the benefit conferred, will find in these cases of transposition of the big claw from one side to the other evidence of the acquirement of a useful process through natural selection, but I do not think that there is any connection of this sort in these cases.[1]

I have intimated above that the injury to the blood-vessels that run to the leg may be closely connected with the changes that take place in the leg, and account for the absence of transposition in those experiments of Wilson's in which the nerve of the small claw was cut (and presumably also the blood-vessel). My work on the fiddler-crab convinced me that cutting the blood-vessels, which seems nearly always to take place when the nerve is cut, brings about important changes in the condition of the leg. If my suggestion prove correct, namely, that the lack of transposition in Wilson's experiment is due to injury to the blood-vessel rather than to cutting the nerve, then it is possible that the whole phenomenon of transposition may be connected with the condition of the blood supply to the leg. After removal of the large claw more blood may be thrown into the vessel going to the small claw, and this may be the cause of the change that takes place.

[1] In this respect I am in entire agreement with Wilson.

NOTES ON REGULATION IN STYLARIA LACUSTRIS.

E. H. HARPER.

This paper is not concerned chiefly with regeneration, but more especially with some processes of regulation accompanying regeneration which were observed in the living animals. *Stylaria lacustris* L. is a well-known fresh-water oligochæte of the family Naididæ, whose members have the power of reproducing by self-division. The worm is six to eight mm. in length, and is easily recognized by its long prostomium or proboscis. The body is divided into three regions: (1) An anterior specialized region of five segments, containing the pharynx, which is marked by a yellow pigment distinct in appearance from the dark brown chloragogue layer of the rest of the alimentary tract. The first segment bears the long prostomium and eyes. The mouth is ventral and the pharynx is slightly eversible. The anterior region is further distinguished by the absence of the capilliform dorsal setæ, ventral setæ also being absent from the first segment. (2) The middle region, with an indefinite number of segments, containing the œsophagus and the crop, a dilatation between the seventh and eighth segments, and the stomach-intestine. The middle region ends in a budding zone of incompletely developed segments and embryonic tissue. (3) The specialized anal segment, somewhat longer than the rest and tapering in form, devoid of setæ, and with the gut lined with cilia.

Self-division takes place at a region posterior to the middle of the worm, from the seventeenth to the twenty-fifth segments, very commonly at the end of the twenty-first. The first indication of division is the appearance of a band of transparent tissue divided in the middle by a slight constriction. Embryonic tissue accumulates before and behind the septum between the segments forming an ectodermal thickening. These regions lengthen and soon become segmented, that in front of the septum forming the anal segment of the anterior zoöid, and the part behind developing into a pharyngeal region of five segments for the posterior zoöid. The zoöids remain united until completely

developed, a continuous band of fæcal matter being visible through the length of the worm. During rapid division another zone of fission makes its appearance, one segment in front of the one previously formed, making a chain of three zoöids.

This method of self-division is often compared with the more primitive type called fragmentation in which the parts separate before regenerating the ends. The physiological regeneration that takes place in self-division is the same in its results as regeneration from a cut surface. Asexual multiplication continues through the warmer months. In October and November the sexual organs attain maturity. Budding then ceases, and the power of regeneration is also diminished.

Methods.

Whole mounts of these worms have been made by the following method. As stated above, the results of this paper have been obtained from the study of the living forms, but when it has been desired to preserve specimens to show results of regeneration, difficulty was encountered in killing the animals in an extended condition, since they invariably become coiled up on application of the fixing fluid. A remedy for this was found by getting the animal extended in the angle between the beveled edge of a slide and a glass plate. A very little hot sublimate-acetic applied to the animal in this position will be drawn under the slide with considerable force and prevent the animal from moving. If as little liquid as possible be used, the animals may be killed in a perfectly extended condition. The animal should be placed on the glass plate in a small drop of water. If the slide is then moved up to the drop, the worm will become extended next to the glass, and held there with sufficient force to prevent its coiling when the killing fluid is applied.

I. Regeneration in the Asexual Forms.

Experiments. 1. A résumé of some general features of regeneration in *Stylaria.*

These forms including *S. lacustris* have been studied extensively, both in respect to their method of self-division and their regeneration. Section within the middle region is followed by

regeneration of the pharyngeal region of five segments from an anterior cut surface. Similarly, the anal segment is regenerated from a posterior cut surface, but within a shorter time. In regeneration a bud or knob of transparent embryonic tissue is first proliferated, which increases in length, develops a prostomium, and becomes segmented within a few days. Section within the anterior specialized region is followed by the restoration of the number of segments which were removed, from one to five.

Thus it is seen that the pharyngeal region of five segments is the unit of regeneration after section within the trunk region, and the process is identical with the normal or physiological regeneration that occurs in self-division. On the other hand, the segments are the units of regeneration when the mutilation is within the pharyngeal region.

There is a close analogy between these results and the observations made upon the earthworm, in which four or five segments are regenerated after more than five are cut off.

The prostomium has great power of regeneration. Frequent cases are met with of forms with regenerating prostomium. Child ('00) figured a specimen found in nature with a forked prostomium which was doubtless a product of regeneration. In Fig. 9 is shown a somewhat similar case of regeneration of the prostomium following the severing of the organ at the base; in this case the organ is doubled. In later stages the lateral bud was absorbed.

2. Regulation of the intestine behind a cut surface to form the œsophagus and crop.

It was stated at the outset that this paper deals chiefly with certain processes of regulation accompanying regeneration proper. As an excellent example of the general regulative changes accompanying regeneration in this worm a case of regulation may be mentioned which occurs normally in the process of self-division or physiological regeneration as well as in cases of regeneration after injury. The œsophagus and crop occur in the sixth to eighth segments, *i. e.*, not in the region which is formed from new tissue by proliferation. Hence the œsophagus and crop must be formed by a process of regulative transformation of the intestine behind the new proliferating tissue. This involves a

narrowing of the intestine to form the œsophagus and a dilatation to form the crop. The histological features are not considered here. The same process occurs in regeneration from a cut through the intestine.

3. Inhibition of the zone of fission under the influence of a regenerating region anterior to the fission plane.

A striking instance of regulation is seen in the effect produced upon a zone of fission by section anterior to it. Under certain conditions the formation of a regenerating region will inhibit the process of self-division and cause the disappearance of the zone of fission. This involves the rearrangement of tissues and a process of redifferentiation in a region at a distance from the cut surface. If the animal is severed in front of and near to a zone of fission which is in an early stage of development before segmentation has taken place, the constriction and the accumulated embryonic tissue disappear and the zone is completely redifferentiated (Fig. 1, *a–c*).

The nearer the regenerating region is to the zone of fission the greater is the tendency for the latter to disappear, but the influence may be exerted at a considerable distance. The following table gives the results in 48 cases:

Number of Segments Between the Cut Surface and the Zone of Fission.	Number of Experiments.	Disappearance of Zone of Fission in.
1–7	28	60 per cent.
8–15	20	15 per cent.

In all the cases included in the above table the zone of fission was in an early stage of development, but not all were at exactly the same stage. If only specimens were taken for experiment in which the zone was just beginning to form, no doubt a much larger percentage of cases of disappearance would be obtained both when the regenerating region was near and when it was far away.

The writer was informed by Professor Frank Smith of an observation upon a nearly related form, *Pristina* sp., to the effect that the zone of fission may disappear in animals kept in the laboratory, as a result of insufficient food or other unfavorable conditions. It is realized that a control experiment would be desirable, to show what percentage of cases of disappearance of

the fission zone might be traceable to other causes than the influence of the regenerating region. The results here given are, however, relied upon in the belief that the early disappearance of the zone of fission (in all cases in about two or three days) sufficiently indicated that it was due to the influence of the regenerating region. There is also the evidence of the cases to be mentioned later in which the zone of fission did not disappear subsequent to a cut behind the zone of fission.

An effect is produced the converse of what has just been described when an animal is severed in front of and near to a well-advanced zone of fission. In such a case its development exerts a retarding influence on the regenerating region. A small piece in front of such a zone of fission is soon separated, after undergoing little or no regeneration.

4. Effects of section behind a recently formed zone of fission.

Section behind a recently established zone of fission does not ordinarily seem to exert an inhibitive influence. In only one such case observed was the zone redifferentiated. No change is produced, unless it be a retardation of the development of the zone of fission. Two considerations seem to have weight in explaining the difference between this and the case in which the cut is anterior to the fission plane. First, the regeneration of the anal segment is a process requiring the withdrawal of less material from the old parts than the regeneration of an anterior end, and hence is calculated to interfere less with the development of the zone of physiological regeneration. Second, the establishment of a regenerating region behind the zone of fission does not materially change the conditions already existing in that region, since in this case a regenerating region posterior to the fission plane is substituted for the previously existing proliferating region in front of the anal segment.

Galloway ('99) has noted in respect to *Dero vaga* that cutting behind the fission zone tends to disorganize its growth and the posterior piece soon drops off.

As a parallel to the results of the preceding experiments upon *Stylaria* it may be mentioned that Dr. Child has informed me that he has found a similar disappearance of the fission plane in the Rhabdocœle *Stenostoma*, under the influence of a regenerating

region. There is probably a close similarity in the physiological processes connected with fission in the two groups.

5. Disorganization in the region behind a cut surface.

(*a*) There are a number of phenomena taking place internally during regeneration which can be plainly seen, owing to the transparency of these animals. When a portion of the head segment in front of the eyes is severed, in almost all cases the pigmented portions of the eyes are found later in a fragmented condition and the black pigment cells scattered through the pharyngeal region (Figs. 2, 5, 11). This does not seem to be a direct mechanical effect, since it occurs when the eyes are apparently not directly injured by the mutilation. This observation, which may seem of slight importance, is mentioned as showing how organs behind the regenerating region may be affected, apparently disorganized, in this case, and later restored. The eyes are reformed subsequently and the scattered pigment cells disappear. In Figs. 5 and 11 the eye appears in two parts. It became normal later. The interpretation, if such it may be called, which would seem to be indicated by the facts, is that the region just behind the cut surface tends to return to an embryonic or disorganized condition as the result of change in physiological conditions.

(*b*) Another observation of a like nature has been made in the case of the regeneration of the whole pharyngeal region. During the regeneration numerous brown pigmented cells, similar to the chloragogue cells of the stomach-intestine, are seen floating in the cœlomic fluid. These are different from the normal unpigmented cœlomic corpuscles. As was pointed out above, the region behind the cut surface is a seat of regulative activity, the œsophagus and crop being produced in that region by regulation of the intestine. The free pigment cells may be a product of the disorganization of the intestine immediately behind the cut surface, as in the case of the pigment cells of the eye.

As stated above, these two observations seem to indicate that the organs just behind the cut surface tend to return to an embryonic condition similar to that of the new proliferated material. This breakdown of tissues may also be comparable to the loss of cell boundaries and return to a syncytial condition in the well-

known experiments of Loeb with segmenting *Fundulus* eggs, in which the cause ascribed was lack of oxygen.

6. Healing of a cut surface without regeneration.

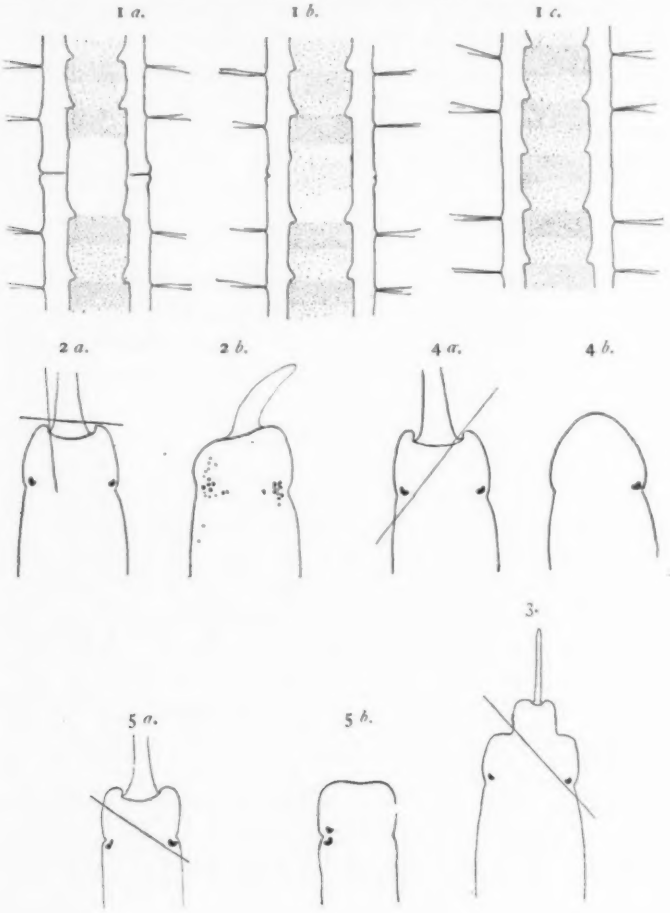
The regenerative power is so strong in the asexual naïds that it would naturally be expected that regeneration would take place under all circumstances in pieces above a certain limit of size. Certain instances of failure to regenerate have been met with, however, which indicate that the power to regenerate is dependent upon certain internal conditions. If an oblique cut be made in front of the pharynx, removing the prostomium and one side of the head segment, including perhaps one eye, the cut surface may frequently heal over and failure to regenerate the prostomium may result (Figs. 4 and 5). A number of such cases have been obtained. In some instances the animals were kept for more than three weeks without showing any tendency to regenerate the prostomium. Since regeneration ordinarily requires only a few days, the effects seemed in these cases to be permanent.

Internal conditions favorable to proliferation, such as the exposure of cut surfaces of intestine and blood-vessels, are present in nearly all possible experiments with *Stylaria*. But after section of the prostomium and one side of the head without touching the pharynx, the tendency to proliferate is apparently at a minimum. The ectoderm may, therefore, close over the wound and cut off the outlet for proliferating material. Failure to regenerate seemed to follow only after an oblique cut, but in one instance, after a transverse cut in front of the eyes, a somewhat similar result was obtained. In this animal an outgrowth appeared from the cut surface which was of less diameter than the region behind, appearing like a new head segment superimposed upon the old. Its appearance suggested that the outlet for the proliferating material had been narrowed by reduction of the cut surface. It is conceivable that still further reduction of the cut surface, or even complete closing of the wound, might occur in case the proliferating tendency was slight (Fig. 3).

The above cases of failure to regenerate were only occasional instances, showing that the balance between the tendencies to proliferate and to heal over without regeneration is rather deli-

cately adjusted. Cases occurred in which the prostomium was regenerated, but the side of the head or lobe was not normally restored, at least within the period of observation (cf. Fig. 13, of a sexual individual).

The general conclusion is that certain parts which may be mutilated without exposing cut surfaces of internal organs which are of importance as internal factors in proliferation are less likely to be regenerated, since, when the proliferating tendency is slight, the ectoderm closes over the wound and checks proliferation.



DESCRIPTION OF FIGURES.

FIG. 1. (*a*, *b*, *c*.) Redifferentiation of a zone of fission. In (*a*), a zone is formed and there is a constriction in the body-wall. The intestine in this region is transparent, devoid of pigment. In (*b*) and (*c*) the zone and constriction have disappeared and the intestine becomes pigmented. The accumulation of transparent embryonic tissue before and behind the septum in (*a*), mentioned in the text, is not indicated in the figure. The ectodermal thickening is indicated at the constriction. In this and all the figures the dorsal capilliform setæ are not represented full length.

FIG. 2. Fragmentation of the eyes after mutilation. The prostomium was severed and a short longitudinal cut made in the first segment. The direction of the cut indicated by line. The condition in (*b*) was observed three days after the mutilation. The slightly curved outgrowth of the bud of the prostomium is an unusual occurrence.

FIG. 3. Regeneration from first segment, resembling a repetition of the head segment, of less diameter. The line indicates direction of the cut. The eye is regenerated. The condition here represented was fifteen days after the operation.

FIG. 4. Failure of regeneration after an oblique cut through first segment. In (*b*) the condition twenty-three days after the operation is shown. The eye is not regenerated and there is no trace of a prostomium.

FIG. 5. Failure of regeneration in first segment. The eye in (*b*) is fragmented into two portions. Later it became normal.

7. Absence of regeneration in short posterior pieces.

With the purpose of finding whether heteromorphosis would take place, as in the earthworm, in the case of very short posterior pieces a number of experiments were tried with as short posterior pieces as would survive the operation. No tails appeared on the anterior ends. A number of pieces which survived the operation for as long a time as three weeks, did not regenerate the anterior ends. During the first week these pieces elongated within the budding region in front of the anal segment. The immature segments became further differentiated, their setæ increasing in size, but no new segments appeared. The pieces then appeared to be composed of segments nearly equal in size, and there was no zone of embryonic undifferentiated tissue remaining. One such piece was obtained by a cut one segment behind the pharynx of a posterior zoöid. It contained at first a region composed chiefly of indistinct segments. At the end of a week these segments had increased in length and their setæ had grown until twenty segments could be easily counted. Its size was greater than that of a minimal piece capable of regeneration taken from the middle of the body. It showed no trace of anterior regeneration for three weeks, but was still active in its movements. Un-

fortunately, it was not possible to keep it under observation longer. A number of similar pieces have been kept with the same results (Figs. 6 and 7). The elongation of such pieces did not seem to be merely mechanical, since it was accompanied by some differentiation such as the growth of the segments. Possibly the proliferating tendency at the cut surface was checked by the continued growth of the budding zone. The latter, containing an accumulation of embryonic tissue, was perhaps able to check regeneration by absorbing the available materials for growth. Whether such pieces would in time regenerate needs, of course, to be determined before the possibility of heteromorphosis would be settled.

8. Regeneration of the posterior end.

It was thought possible that heteromorphosis might occur in regeneration from short anterior pieces. In no case did the specialized anterior region of five segments survive long enough to begin regeneration. An anterior piece of eight segments survived for six days without showing any posterior regeneration. This was the shortest piece which was able to survive more than a short time after the operation. Pieces with only two more of the trunk segments present on the average regenerated freely, however, as the following table will show:

Length.	Number of Experiments.	Number Able to Survive for Several Days.	Number Able to Regenerate.
8–9 segments.	9	2	0
10–11 "	10	9	7
12–13 "	10	9	9
14–29 "	37	35	33

There is a great average increase in the power to regenerate correlated with the presence of only two additional segments of the trunk. The specialized head region, as would be expected, has the least power of regeneration. Portions of the trunk of the same length as the first class given in the above table are able to regenerate freely.

No cases of true heteromorphosis were obtained. The anterior region of five segments which might perhaps be expected to develop a head posteriorly, contains no nephridia (they are found back of the sixth segment), and as we have seen, does not survive the operation long enough to regenerate. Results obtained in

Planaria, with its diffuse excretory system, could not be expected to occur in a form like *Stylaria*, with a head region so specialized as to be unable to exist independently even for a short time.

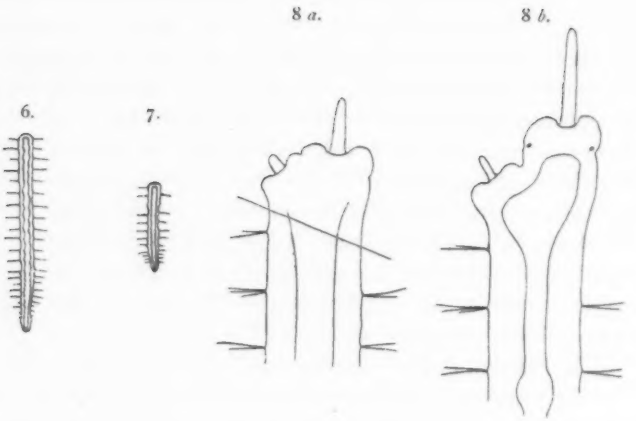
A case of duplication of parts was seen in the production of a double-headed individual, one of whose heads was larger and in line with the axis of the trunk. The other was lateral, lying close to it, and smaller. The smaller one was in process of absorption at the last stage at which it was observed (Fig. 8).

A doubling of the prostomium was noticed in one case as mentioned above. The two organs lay parallel and were fused together except at the tip of the shorter (Fig. 9). In later stages the lateral bud was absorbed.

9. Regulation of growths oblique to the main axis.

It is a common observation that proliferation tends to take place along an axis normal to the cut surface. After an oblique cut the new growth continues oblique until it has reached its full length or nearly so. The straightening of such oblique growths is evidently not a function of growth alone, but of growth influenced by certain tensions, which may be exerted by the part behind the new growth. Rievel ('96) has pointed out the influence exerted by the peristaltic motions of the intestine upon the regeneration of the head region. The knob of new tissue is at first solid and is later penetrated by the slower ingrowth of the lumen of the intestine into the region. The waves of peristaltic motion constantly passing along the intestine favor its ingrowth into the new region. Finally the mouth is formed by the bulging out of the body-wall, caused by peristalsis. It is broken through from within, there being no stomodæal invagination of the ectoderm in regeneration.

It may be suggested that peristaltic movements of the intestine have an influence in straightening oblique growths. This is probable since the straightening occurs not during the proliferating period, but coincidently with the ingrowth of the pharynx. Straightening occurs, moreover, while the tissue still appears embryonic before the complete differentiation of muscular and nervous systems (Figs. 10 and 13).


8 a.
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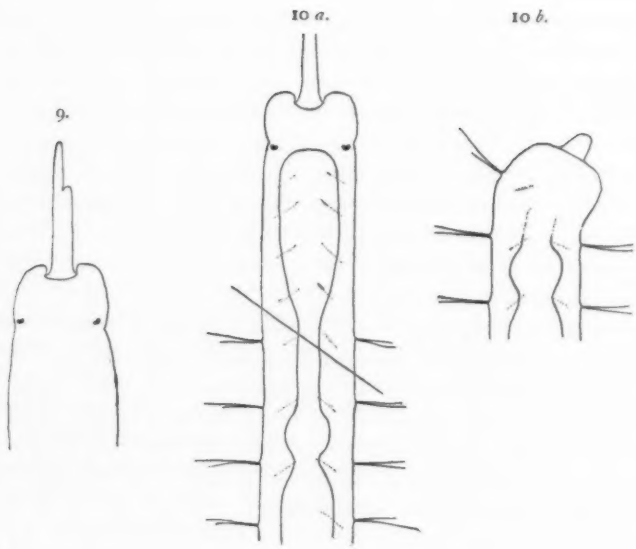
6.
7.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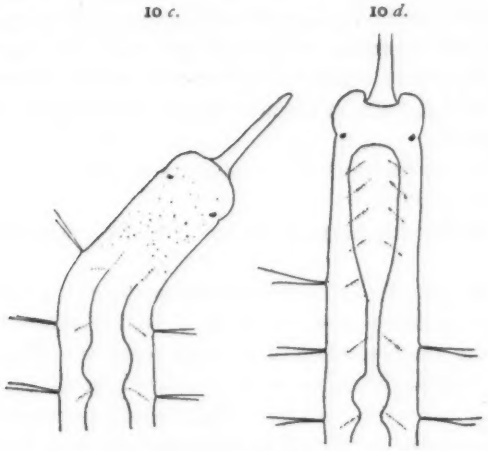


DESCRIPTION OF FIGURES.

FIGS. 6 and 7. Failure of anterior regeneration in case of very short posterior pieces. Fig. 6 represents such a piece twenty-three days after it was severed, Fig. 7 a piece eighteen days after it was severed. It measured 1.25 mm. Further observation was prevented.

FIG. 8. Regeneration of a double-headed individual. The line in (*a*) indicates direction of cut. The smaller head was apparently destined to be absorbed at last observation. The specimen was lost before regeneration was complete. Stages (*a*) and (*b*) were six and ten days after the cut was made.

FIG. 9. Regeneration of abnormal prostomium, a doubling of the organ. Later the lateral bud was absorbed.

FIG. 10. Regeneration occurring in a direction normal to the cut surface. (*b*) represents the condition three days after the operation, (*d*) the complete regeneration. In (*d*) the right dorsal bundle of setæ is not restored. In (*c*) scattered pigment cells resembling the chloragogue cells are present in the new tissue.

II. REGENERATION OF THE SEXUAL FORMS.

The sexual organs of *Stylaria* come to maturity during the months of October and November. In the height of the sexual stage the animals cease to multiply asexually. In the earlier stages budding animals are frequently seen. In the fully developed sexual animal the formation of new segments in the anal proliferating region also ceases. As a result the animals attain larger size, since the segments in the budding region become fully differentiated. The clitellum is conspicuous, covering the fifth and sixth segments, in which the testes and ovaries lie. This part becomes more opaque and loses its setæ.

The results obtained in regard to the regeneration of the sexual forms will be treated under two heads: (1) The regeneration of the pharyngeal region involving the results following the severing of 1–5 anterior segments; (2) regeneration within the trunk region.

1. In the regeneration of the pharyngeal region three sorts of cases are met with:

(*a*) Complete regeneration may occur in the less mature sexual forms.

(*b*) There may be an incomplete regeneration, resulting in the production of an outgrowth which is devoid of ventral setæ and seems to be the equivalent of the first segment. The regenerated part is distinguishable by its relative lack of pigment and by the fact that it does not attain the original diameter (Fig. 11). It is comparable to Fig. 3, where a growth took place in an asexual form from an apparently restricted outlet, and the same explanation may be offered here, viz., that owing to the slowness of proliferation the partial closure of the cut surface by the body-wall restricted the outlet. The prostomium does not reach full length and the lateral lobes of the head are not developed. Often the eyes are not formed in such cases.

(*c*) Often complete failure to regenerate occurs. The end heals over and a mouth is formed (Figs. 12 and 14). In twenty-one cases of mature sexual animals in which from one to four segments were removed there were six such cases of failure to regenerate and the rest regenerated an amount apparently equivalent to one segment.

2. After the removal of five or more segments results followed which likewise may be divided into three classes.

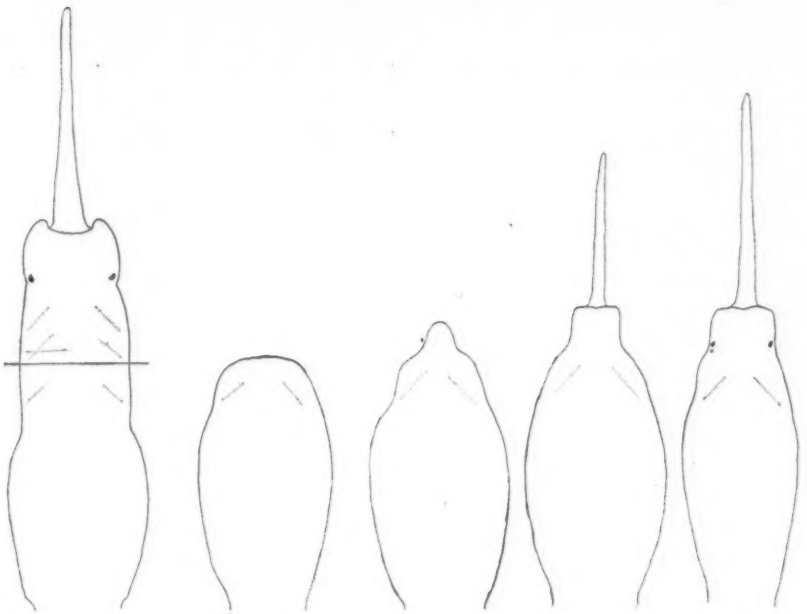
(*a*) Normal regeneration in the less mature sexual specimens.

(*b*) A pharyngeal region of five very short segments with a diminutive prostomium may be formed. Four pairs of ventral setæ appear showing that the full number of segments is represented, but the region is remarkable for its dwarfed appearance (Fig. 15).

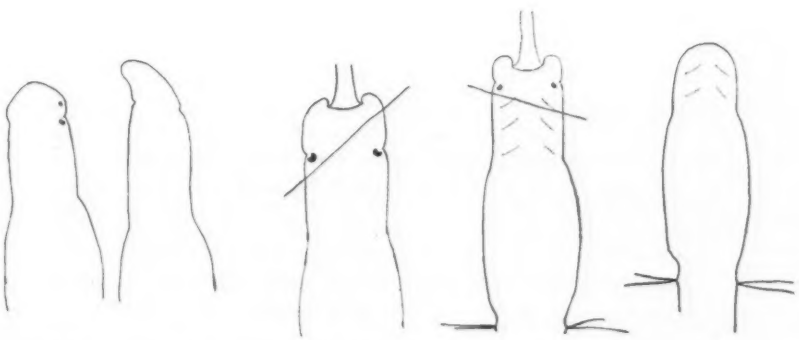
(*c*) There may also be failure to regenerate. In twenty-nine cases in which from six to thirty segments were removed there were six cases of failure to regenerate anteriorly and five cases of the dwarfed regeneration.

The regeneration of the anal segment is equally slow in the advanced sexual forms and the part regenerated is not of full size (Fig. 16).

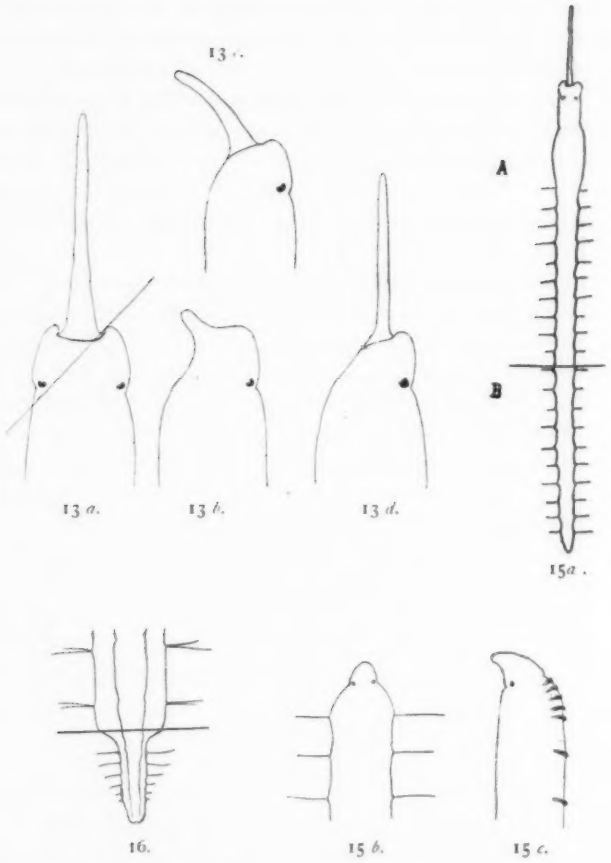
3. In a single instance the clitellum was regenerated, but the specimen was unfortunately not kept under observation long



11 *a*. 11 *b*. 11 *c*. 11 *d*. 11 *e*.



12 *c*. 12 *b*. 12 *a*. 14 *a*. 14 *b*.



DESCRIPTION OF FIGURES.

FIG. 11. Regeneration of a sexual individual after removal of first three segments. The clitellum is indicated by the widening of the body. It takes in the fifth segment, which is a part of the pharyngeal region. The setæ are absent from the clitellum. One pair of ventral bundles were left in front of the clitellum. In stage (*e*) the head segment is regenerated. As no more pairs of setæ bundles were produced, the regenerated part is regarded as the equivalent of the head segment alone. The stages (*b*–*e*) followed 3, 8, 12 and 19 days after the operation, respectively.

FIG. 12. Failure of regeneration after severing part of first segment in a sexual individual. In (*b*) a side view is given. The condition shown in (*b*) and (*c*) followed seventeen days after the cut.

FIG. 13. Oblique growth of bud of prostomium in (*b*), and slightly incomplete regeneration. Stages (*b*, *c*, *d*) were 4, 8 and 18 days after the operation, respectively. The eye was not regenerated. The other eye increased in size.

FIG. 14. Failure of regeneration in a sexual individual after removal of two segments. This condition existed nineteen days after cut was made.

FIG. 15. Dwarfed anterior regeneration from a sexual individual after removal of fifteen segments. The prostomium is not regenerated. A pharyngeal region of five short segments is produced, as shown by the number of setæ bundles. In (*c*) a side view is given. The level at which the cut was made is shown in (*a*).

FIG. 16. Regeneration from posterior end of a sexual individual. The part produced is of less diameter.

enough to determine whether the regeneration of the sexual organs was complete. The cut was made just behind the clitellum.

CONCLUSIONS.

1. The formation of a regenerating region will under certain conditions inhibit the process of asexual multiplication and cause the disappearance of the zone of fission. This effect may be produced by a cut anterior to the zone of fission, less often by a cut posterior to it, and occurs only when the zone is embryonic. The zone is also more likely to disappear if the cut is near to it. The band of transparent embryonic tissue redifferentiates and the energy of growth is transferred to the regenerating region.

2. There is evidence of disorganization and a return to embryonic conditions in organs just behind a cut surface. This effect does not appear to be a direct mechanical result, *i. e.*, due to crushing. Fragmentation of the pigmented portion of the eye is one case adduced.

3. Internal conditions favorable to proliferation, such as the exposure of cut surfaces of intestine and blood-vessels, are present in nearly all possible experiments. But if a corner of the head segment be removed, including the prostomium, without injuring the pharynx, the ectoderm may close over the surface and regeneration may fail to take place.

4. Short posterior pieces often fail to regenerate anteriorly, but no cases of heteromorphosis, such as individuals with tails on the anterior end, have been obtained. Short posterior pieces which failed to regenerate within the time of observation may elongate and show some differentiation in the budding region.

5. The middle portion of the body has the greatest power of regeneration, the specialized pharyngeal region has the least.

6. Growth takes place at right angles to a cut surface, and if the cut is oblique the bud will grow out at an angle to the axis of the body. Straightening is affected after the penetration of the lumen of the pharynx into the region, probably under the influence of the tension produced by the peristaltic motions of the intestine.

7. Sexual individuals lose the power of regeneration to a large extent just as they do their power of budding. In an advanced sexual stage regeneration may fail to occur or be incomplete.

I wish to express my thanks to Dr. C. M. Child for suggesting to me the subject of this paper and for further suggestions relative to some of the results described.

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VARIABILITY IN THE NUMBER OF TEETH ON THE CLAWS OF ADULT SPIDERS, SHOWING THEIR UNRELIABILITY FOR SYSTEMATIC DESCRIPTION.[1]

CARL HARTMANN.

Since the number of teeth on the claws of spiders is often used as a specific character, the need of testing its constancy suggested itself. It has been pointed out by W. Wagner[2] that the number of ungual teeth varies with each moult. In the present study of the variations in the adult this fact was well taken into consideration, great care being exercised in choosing fully mature individuals that had undergone the last moult. To my knowledge no one has ever tested the constancy of the number in fully mature individuals.

The study was made on the claws of the right legs of 70 females and 40 males and comprises, therefore, observations on nearly 1,320 claws of 440 legs. Representatives of a number of different families were chosen as follows: Dictynidæ (*Dictyna volupis*, Keys., West Chester, Pa.), Theridiidæ (*Theridium tepidariorum*, Koch, Philadelphia), Pholcidæ (*Spermaphora* sp.? Hentz, Austin, Texas), Epeiridæ (*Epeira marmorea* Clerck; *E. benjamini*, Walck.; *Acrosoma reduvianum*, (Walck), West Chester, Pa.), Lycosidæ (*Lycosa nidicola*, Emerton, Austin, Tex.).

The counting of the claws was easy except in the case of *Dictyna volupis* and of *Spermophora;* but even here, if any mistakes in the counting are recorded, they are extremely few, for I never left a claw until convinced that my count was correct; or in a few cases where this seemed impossible the individual was entirely discarded. To count the teeth the three claws of each leg, if they were large, were snipped off with a needle (keeping the foot on a slide in a drop of alcohol) and pressed flat with a cover-glass. If the claws were too small, the whole tarsus was

[1] Contributions from the Zoölogy Department of the University of Texas, No. 55.

[2] W. Wagner, "La Mue des Aragnées," *Ann. Sc. Nat.*, 1888, p. 363.

Table I. Females.

Species	No. of Leg.	No. of the Individual.									
		1	2	3	4	5	6	7	8	9	10
Dictyna volupis.	1	13,9 5	13,12 5	13,11 5	12,11 5	12,11 5	12,10 5	14,12 5	14,12 5	12,12 5	13,11 5
	2	12,10 5	——	12,12 5	12,10 5	11,— 5	12,12 4	12,12 5	12,11 5	13,13 5	11,12 5
	3	9,10 4	10,9 4	–,9 4	9,9 4	9,9 3	8,9 4	9,10 3	10,10 4	10,10 3	9,9 4
	4	9,10 4	9,10 4	10,10 4	9,10 4	9,10 3	9,9 4	11,10 4	9,10 4	10,11 4	10,10 4
Theridium tepidariorum.	1	6,6 2	6,5 2	5,8 2	6,5 2	6,5 2	6,6 2	4,5 4,5 2 2	6,5 –	6,5 2	6,6 2
	2	6,6 2	6,5 2	6,5 3,2	6,5 2	6,6 2	6,5 2	5,5 –,6 2 2	7,5 2	6,6 2	6,6 2
	3	5,6 2	5,6 2	5,5 2	5,6 2	5,6 2	5,6 2	–,5 –,6 2 2	5,6 2	5,6 2	6,6 1
	4	4,2 2	3,2 1	4,2 1	4,2 2	4,2 2	4,1 1	3,2 3,2 1 1	4,2 1	3,1 2	4,2 1
Spermophora.	1	11,10 1	10,10 1	11,11 1	10,10 1	10,10 1	10,10 1	11,10 1	11,10 1	9,9 1	11,10 1
	2	10,10 1	10,10 1	11,10 1	——	12,10 1	10,10 1	11,10 1	11,11 1	9,9 1	10,10 1
	3	9,10 2	10,10 1	11,11 1	10,10 1	11,11 1	9,9 1	10,9 1	10,10 1	9,9 1	9,12 1
	4	7,9 0	7,9 1	–,9 2	9,10 1	7,9 1	8,10 1	8,10 1	8,9 1	7,8 1	10,10 1
Epeira marmorea.	1	7,8 2	8,8 2	7,8 2	8,9 2	9,7 2	7,8 3	7,8 3	8,8 2	8,8 3	9,9 3
	2	7,8 2	6,7 2	7,8 2	9,9 2	8,8 2	8,7 2	7,8 3	7,8 2	8,8 2	8,8 3
	3	6,6 2	–,7 2	——	6,7 2	7,6 2	6,5 2	6,6 2	6,7 1	6,6 2	8,7 2
	4	——	5,6 2	6,5 1	6,8 2	6,6 2	5,6 2	6,5 2	6,5 2	6,6 2	6,7 2
Lycosa viduola.	1	4,4 0	4,4 0	4,4 0	4,4 0	4,4 0	5,4 0	5,4 0	4,5 0	4,4 0	4,4 0
	2	7,6 0	7,6 0	5,4 0	5,4 0	5,4 0	6,5 1	5,4 0	5,6 0	5,5 0	4,5 0
	3	——	7,6 0	6,6 0	7,6 0	6,5 0	7,7 0	7,6 0	7,7 0	7,7 0	6,6 0
	4	6,5 0	9,9 0	6,7 0	6,7 0	5,7 0	8,8 0	7,7 0	8,8 0	7,7 0	7,7 0
Epeira benjamini.	1	5,8 2	5,9 2	8,– 3	8,8 2	9,8 3	8,8 2	10,8 3	8,9 2	7,8 2	6,8 2
	2	5,9 2	6,9 2	5,6 3	7,9 2	7,9 3	8,8 2	8,9 2	7,8 2	6,8 2	7,8 2
	3	3,6 2	8,7 2	7,6 2	7,7 2	8,7 2	7,7 2	6,11 2	7,6 2	7,7 2	6,6 2
	4	6,5 2	7,6 2	7,6 2	4,5 2	6,5 2	5,5 1	6,5 2	6,5 2	6,5 2	6,5 2

TABLE I. FEMALES.—*Continued.*

Species	No. of Leg.	No. of Individuals.									
		1	2	3	4	5	6	7	8	9	10
Acrosoma rediviuum.	1	7,6 2	7,6 2	7,7 2	7,6 2	7,7 2	7,6 2	8,7 2	7,6 2	7,7 2	7,7 2
	2	——	7,6 2	7,6 2	7,6 2	7,– 2	6,6 2	6,7 2	7,7 2	7,7 2	6,7 2
	3	5,6 2	5,5 2	6,5 2	6,5 2	6,6 2	5,5 2	6,6 2	5,5 2	6,6 2	5,5 2
	4	3,2 1	3,4 2	3,4 2	4,4 2	——	4,5 2	5,4 2	4,2 1	4,4 2	4,3 1

covered, placed under the compound microscope and pressure applied until all the teeth came into full view.

The results of the counting of the teeth are recorded in the accompanying tables, Table I. containing the figures for the females and Table II. for the males. The three claws were always distinguished from one another and the number of teeth on each recorded after the formula $\frac{a, p}{i}$, where a represents the number of teeth on the anterior, p on the posterior and i on the inferior claw.

The reduction in the number of teeth seems to take place at the proximal end of the claw because, firstly, the distal teeth usually maintain the size and form characteristic of the species, and secondly because the proximal tooth (or teeth) in some cases becomes so small as to merit the name tubercle in place of tooth. This latter fact forced me to establish a criterion to determine what to count as a tooth and I decided to call the structure a tooth if it had attained a length at least half as great as its width at the base. In *Dictyna volupis* the two distal teeth are small and are closely approximated to the claw for nearly their entire length. One of these was counted in some four or five cases where it was unusually large and stood out from the claw for at least half its length.

In order to reduce the tables to percentages so as to get at a simple set of figures for comparison I have adopted what may be called the "percentage of constancy" method, which may be illustrated as follows:

In Table I. the anterior claw of the first leg of *Lycosa nidicola*

TABLE II. MALES.

Species	No. of Leg.	No of the Individual									
		1	2	3	4	5	6	7	8	9	10
Dictyna volupis	1	12,11 5	12,11 5	12,10 5	12,11 5	12,9 5	11,11 5	11,11 5	10,10 4	11,11 7	11,10 6
	2	12,12 6	12,11 5	13,12 5	11,11 5	12,11 5	12,11 5	11,– 5	11,11 5	12,11 6	11,10 6
	3	11,11 4	11,10 4	9,9 4	9,10 4	9,9 4	9,9 4	10,10 5	9,9 4	–,10 4	10,10 5
	4	11,11 5	10,10 4	9,9 4	10,10 4	10,9 4	10,10 4	11,10 4	9,10 4	10,11 5	10,10 5
Theridium tepidariorum	1	4,4 1	5,5 1	5,4 2	5,5 2	6,4 2	–,4 2	5,– 2	5,5 2	5,5 2	5,5 2
	2	4,4 1	5,4 2	5,4 2	5,4 2	5,4 2	5,4 2	5,4 1	5,5 2	5,5 2	5,5 2
	3	–,4 1	4,5 2	5,3 2	4,5 2	3,5 2	4,3 2	4,5 2	4,3 2	4,4 2	5,5 2
	4	2,1 1	3,1 1	3,1 1	3,1 1	4,1 1	3,– 1	4,1 1	4,1 1	3,1 1	4,1 1
Sphermophora	1	12,11 1	12,– 1	12,11 1	12,10 1	11,10 1	10,9 1	10,10 1	11,10 1	11,10 1	12,11 1
	2	12,10 1	11,10 1	12,11 1	11,10 1	12,11 1	10,9 1	11,10 1	10,10 1	11,10 1	11,10 1
	3	10,11 1	11,11 1	11,10 1	–,10 1	11,11 1	9,9 1	10,10 1	9,9 1	10,9 1	10,10 1
	4	9,10 1	8,10 1	8,10 1	8,10 1	9,10 1	8,9 1	8,9 1	8,9 1	——	9,10 1
Epeira marmorea	1	7,9 2	10,11 2	9,8 2	8,9 2	8,9 2					
	2	8,8 2	7,9 2	8,8 2	8,8 2	——					
	3	6,6 2	6,6 2	7,8 2	6,6 2	6,6 1					
	4	6,6 2	7,– 2	–,5 1	6,5 1	6,6 1					
Lycosa nidicola	1	6,6 0	6,6 0	6,6 0	6,7 0	6,6 0					
	2	7,7 0	8,8 0	7,7 0	7,8 0	7,8 0					
	3	8,9	9,10 1	8,8 0	8,8 0	9,9 0					
	4	8,9 0 0	9,9 0	——	9,9 0	10,10 0					

has 4 teeth 8 times out of 10, the posterior claw has 4 teeth 9 times out of 10. So that the claws have percentages of constancy of 80 and 90 respectively. On the second leg of the same species the anterior claw has 5 teeth more often than any other number of teeth or in other words it has a maximum constancy of

5 in 10 = 50 per cent.; the posterior claw has a maximum constancy of 40 per cent. having 4 teeth 4 times in 10. This method was pursued throughout. Where only five specimens of a species were examined the result was calculated to ten. On the basis of these data the following conclusions were drawn. Where percentages are cited they are percentages of constancy and refer only to the superior claws.

1. Claws having larger numbers of teeth show more variation in numbers than claws having a smaller number and this holds for both sexes. The percentages of constancy for the superior claws of male and female are as follows: *Spermophora* 52 per cent., *Dictyna volupis* 54 per cent., *Epeira marmorea* 59.5 per cent., *Lycosa nidicola* 62 per cent., *Theridium tepidariorum* 68 per cent. A glance at the tables will convince one that this order will practically hold for the relative total number of teeth.

The same result is more strikingly shown by the inferior teeth. In *Dictyna volupis*, which has normally 4–5 teeth on the inferior claw, there are 16 variations in 80 cases of both males and females; while *Spermophora* and *Lycosa nidicola*, which have normally only 1 and 0 tooth respectively have only 3 variations in 80 cases in the former case and 1 in 60 in the latter.

Moreover, the superior claws, having many teeth, show many times as much variation as the few-toothed inferior claw.

2. The teeth on the claws of the first leg show least variation in number, those on the third most. The percentages of constancy are: First leg, 61.5 per cent.; second leg, 60.0 per cent.; third leg, 57.0 per cent.; fourth leg, 58 per cent.

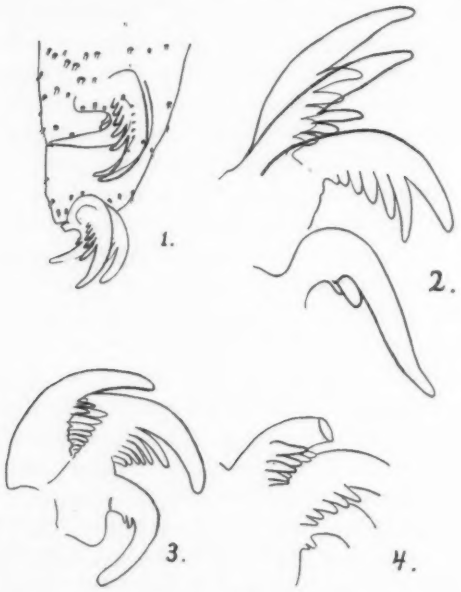
3. The number of teeth on the claws of the female varies slightly more than in the male, the percentages of constancy being 58.0 and 59.75 respectively. The inferior claw gives the opposite result, however, for here in the females there are 17, in the males 19 deviations from the normal in 160 cases. A glance at the tables will disclose a sexual dimorphism in the total number of teeth which may be greater in the male or in the female or the same in both, according to the species. Thus by actual count 5 females of *Epeira marmorea* have 318, 5 males 335 ungual teeth on the right side; but 5 females of *Lycosa nidicola* have 318 teeth as against 228 for 5 males; while 10 females and 10 males of *Dictyna volupis* have each 1,030 teeth exactly.

4. The anterior and the posterior claws vary in nearly equal measure throughout, the average constancy percentage for the former being 58.0, for the latter 59.75.

5. In only one case were two individuals found that had the same number of teeth on the corresponding claws of all the four legs on the right side (Table II., *Theridium tepidariorum*, individuals 2 and 4).

In view of these facts one would be safe in saying that the number of teeth on the tarsal claws of spiders is too variable to be used as a specific character; it should at least not be used in a diagnosis until its absolute constancy for a given species has first been demonstrated.

In addition to this common variation in the number of teeth on the claws one often meets with additional claws or with double



rows of teeth on the same claw (mutations). Fig. 1 represents the first foot of the seventh specimen of *Theridium tepidariorum* in Table I. Two complete sets of claws, similar to the ones

figured for the first leg, were found on each leg of the animal, both on the right and on the left side. These claws were all of the normal type but possessed relatively fewer teeth than those on the claws of other specimens of the same species.

Fig. 2 represents four claws on the second leg of the second female of *Theridium tepidariorum*, the claw bearing three teeth being an additional inferior one.

Figs. 3 and 4 show on the posterior claws double rows of teeth, the additional row lying close to the normal and more regular one. The claws in Fig. 3 are from the third leg of specimen seven of *Epeira benjamini* and those in Fig. 4 from the fourth leg of specimen four of *E. marmorea*, both in Table I.

In conclusion I wish to thank Dr. T. H. Montgomery for the many specimens which he placed at my disposal and for his kind suggestions in my work.

UNIVERSITY OF TEXAS,
AUSTIN, TEXAS, December 24, 1903.

A BIOLOGICAL EXAMINATION OF DISTILLED WATER.

E. P. LYON.

The accuracy of results obtained in many of the present lines of physiological research depends so entirely on the purity of the chemicals used, including water, that I may be pardoned for publishing an account of work which otherwise would have little significance, the toxicity of metal-distilled water having been repeatedly shown. The results obtained are, however, applicable in a practical way at Woods Hole, where it is difficult to get good distilled water.

Two years ago I arranged an automatic still in which the water was boiled in a block tin vessel but the dry steam received and condensed in glass. It was thought that if the water did not touch metal after condensation, contamination by ions would be avoided. The distillate proved, however, to be decidedly toxic. I could not believe that dry steam carried with it an appreciable quantity of metal and, therefore, decided to find out where the trouble lay.

In these experiments, sea-water was condensed to a known fraction of its volume and then measured portions brought back to the original volume by adding the distilled waters to be tested. Tap-water was also used for comparison; sometimes, without treatment; sometimes, sterilized without boiling; in some experiments a portion was boiled away and the remaining portion used.

To the artificial sea-waters prepared as above equal amounts of fertilized *Arbacia* eggs were added and the development observed. The percentage of plutei developed in the different solutions as compared with natural sea-water was usually taken as a measure of purity. In other cases, the percentage of blastulæ was the standard; or the percentage of segmentation in a given time; or the length of time that any larvæ remained alive. The records of some typical experiments follow:

Experiment 6.—250 c.c. sea-water were concentrated to 75 c.c. To each 15 c.c. of this solution 35 c.c. of the waters named

in column 2 were added. To the dishes were added equal amounts of *Arbacia* eggs in 16–32-cell stage.

No.	Description of Water.	20 Hours.	28 Hours.	54 Hours.
1	Double distilled in glass.	Early gastrulæ. Behind control.	Behind control.	Plutei.
2	Tap.	Blastulæ.	Many going to pieces.	All dead.
3	Tap boiled down one half.	Slightly behind No. 1. But much better than No. 2.	Almost as good as No. 1.	Plutei as well developed as No. 1; not so numerous.
4	Control in sea water.			Advanced plutei.

Experiment 11.—Two liters of sea-water were evaporated to 250 c.c. To 10 c.c. portions of the resulting solution were added respectively 70 c.c. of waters described below. Equal quantities of *Arbacia* eggs were added. Three days later the percentages of plutei were ascertained by first killing the cultures and then making counts in a watch glass marked off into squares.

	Per cent. Plutei.
1. Control, 80 c.c. sea-water.	90
2. Artificial sea-water made from tap-water.	0
3. " " " " " tap-water heated to 60° in closed flask.	0
4. " " " " " distilled water from Metcalf's, Boston.	16[1]
5. " " " " " water boiled in copper without H_2SO_4 but condensed in glass.	32[1]
6. Artificial sea-water made from water boiled in copper with H_2SO_4 but condensed in glass.	78[2]
7. Artificial sea-water made from water distilled wholly in glass; first part thrown away.	66
8. Artificial sea-water made from water distilled in patent automatic metal still.	5[3]
9. Artificial sea-water made from water distilled wholly in glass, with $K_2Cr_2O_7$ and H_2SO_4.	87
10. Artificial sea-water made from water double distilled in glass, first distillates rejected.	90
11. Artificial sea-water made from "Pureoxia" distilled water.	3[4]

Experiment 8.—Two liters of sea-water were boiled down to 400 c.c. Ten c.c. of this concentrated sea-water were added to 40 c.c. of each of the waters listed in column 2. To every dish was added an equal amount of fertilized *Arbacia* eggs on July 10, 1903.

[1] Nearly all small.

[2] Some small; others as good as control.

[3] All very small and imperfect.

[4] All very small and imperfect. Only experiment with this brand.

EXPERIMENT 8.

No.	Description of Water.	Condition of Cultures on Dates Named.					
		July 11.	July 12.	July 16.	July 20.	July 23.	July 28.
1	Tap.	No larvæ.					
2	1st 50 c.c. distilled in glass from 1,000 c.c. tap.	Many gastrulæ, but not so good as No. 6.	Many plutei; not so good as No. 3.	Some alive; not so good as No. 4.	All dead.		
3	2d 50 c.c. ditto.	Ditto.	Not so good as control. 40 per cent. plutei.	Many alive.	Some alive.	All dead.	
4	3d 50 c.c. ditto.	Better than No. 2.	As good as control.		Many alive.	Many alive.	All dead
5	Distilled in glass from tap water, which had been boiled in open vessel.	Fine lot of gastrulæ.	As good as control.	Many alive and lively.	All dead.[1]		
6	1st 50 c.c. distilled from 1,000 c.c. tap + 5 grms. potassium bichromate and 10 c.c. H_2SO_4.	Ditto.	Better than control.	Much better than No. 3 or No. 9.	Many alive; not so good as No. 8.	Many alive.	All dead.
7	2d 50 c.c. ditto.	Ditto.	Ditto.	Ditto.	Ditto.	Ditto.	All dead.
8	Double distilled in glass, first distillate in each case thrown away.	Ditto.	As good as control or better.		Fine lot alive.	Fine lot alive.	All dead.
9	Distilled water from Metcalf, Boston.	Ditto.	About like No. 2.	Many alive.	All dead.		
10	Same as No. 5 + 1 c.c. $\frac{m}{10}$ NH_4OH	No development.					
11	Control, in 50 c.c. sea-water.	Fine lot of gastrulæ.	50 per cent. are good plutei.	Some alive.	All dead.[1]		

[1] Not usual in water prepared in this way. Should have lived longer.

From the above and other experiments I can make the statements enumerated below, which apply, of course, to *Arbacia* only but point to the necessity of caution in using distilled water on other organisms. It is true also that the results are most applicable to Woods Hole conditions and to the tap-water used as a basis of the distilled water there. It is probable that the toxicity is due to ammonia although this was not proven. It is certain from experiments made that *Arbacia* larvæ are very sensitive to that substance.

1. Tap-water is decidedly, although variably, toxic. The toxicity is not lost by sterilization but is greatly reduced by boiling the water for a long time, say until one third has been boiled away. The residue in such cases is less toxic than some distilled waters, particularly commercial brands and that from automatic stills.

2. Water from ordinary automatic stills, whether metal or entirely glass, is toxic.

3. The commercial distilled waters used by me were toxic, often in high degree.

4. In distilling water in the ordinary way from glass, the first one tenth distilled over is decidedly toxic, the second tenth less so, the third tenth still less so. The fourth tenth is of good quality.

5. The best distilled water used was produced by double distilling in glass, the first fourth distilled over in each distillation being rejected.

6. Nearly as good water was produced by single distillation from tap-water to which H_2SO_4 and $K_2Cr_2O_7$ had been added.

7. If a little H_2SO_4 is added to the tap-water to start with, an excellent quality of distilled water may be produced from an automatic still consisting of a copper vessel and glass condenser, the arrangement being such that none of the condensed water touches the metal. This water is practically free from ions or toxic volatile substances. It is much better than water double distilled in glass in the ordinary way, unless in the later case a large proportion of the product be thrown away. Such an automatic still is recommended for use at Woods Hole.

8. It was noted in a number of cases that *Arbacia* lived longer

in an artificial sea-water prepared from a good quality of distilled water than in natural sea-water. It is probable that the volatile toxic substance (ammonia?) exists in sufficient quantity in sea-water to have an appreciable effect.

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